

Applicants: Thomas M. Jessell et al.  
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In the Title:

Please replace the current title with the following title:  
"Dorsalin-1 Polypeptide and Uses Thereof".

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In the specification:

Please amend the specification under the provisions of revised 37 C.F.R. §1.121 as follows.

On page 1, line 2, after the Title and before the Background of the Invention, please delete the paragraph which begins "This application is a continuation . . ." and insert the following amended paragraph:

--This application is a continuation of U.S. Serial No. 08/065,844, filed May 20, 1993, now United States Patent No. 6,333,168, issued December 25, 2001, the contents of which are hereby incorporated by reference into the present application.--

On page 7, lines 3-4, please delete the paragraph which begins "Figure 1 . . ." and insert the following amended paragraph:

-- Figure 1  
Figures 1A and 1B Nucleotide and Deduced Amino Acid  
Sequence of Dorsalin-1 (SEQ. ID No.  
1.)--

On page 15, lines 7-17, please delete the paragraph which begins "(J-L) Nomarski (J) and . . ." and insert the following amended paragraph:

--(J-L) Nomarski (J) and immunofluorescence micrograph (K,L) of

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an [i]-neural plate and floor plate conjugate exposed for 48h to  $3 \times 10^{-11}$  M dorsalin-1<sup>myc</sup>. No Islet-1<sup>+</sup> cells are detected (K) whereas the number of 3A10<sup>+</sup> neurons in the neural plate explant (L) is not obviously different from that in the absence of dorsalin-1<sup>myc</sup>. In figures D and [[G]] J, the dashed line outlines the extent of the neural plate (np) explant.--

On page 20, lines 25-35, please delete the paragraph which begins "Dorsalin-1 may be. . ." and insert the following amended paragraph:

--Dorsalin-1 may be produced by a variety of vertebrates. In an embodiment, a human dorsalin-1 nucleic acid molecule is isolated. In another embodiment, a mouse dorsalin-1 nucleic acid molecule is isolated. In a further embodiment, a chick dorsalin-1 nucleic acid molecule is provided. The plasmid, pKB502, encoding a chick dorsalin-1 was deposited on October 5, 1992 with the American Type Culture Collection (ATCC), ~~12301 Parklawn Drive, Rockville, Maryland 20852~~ 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism--

On page 23, lines 21-29, please delete the paragraph which begins "In one embodiment, the expression vector. . ." and insert the following amended paragraph:

--In one embodiment, the expression vector, pKB501 (with myc epitope), containing chick dorsalin-1 with a myc-epitope was

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deposited on October 5, 1992 with the American Type Culture Collection (ATCC), ~~12301 Parklawn Drive, Rockville, Maryland 20852~~ 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganism for the Purposes of Patent Procedure. Plasmid, pKB 501 (with myc epitope) was accorded ATCC designation number 75320.--

On page 29, lines 21-35, please delete the paragraph which begins "The coding region . . ." and insert the following amended paragraph:

--The coding region of dorsalin-1 was isolated using the two PCR primers ORF-5' (5' TGGAATTCATCGATAACGGAAGCTGAAGC 3'; SEQ ID No. 12) and ORF-3' (5' AGCGTCGACATCGATATTCAGCATATACTACC 3'; SEQ ID No. 13) and cloned into pBS SK-between the EcoRI and SalI sites. To insert the c-myc epitope (EQKLISEEDL; SEQ. ID No. 18) two internal primers, each encoding half of the c-myc epitope and dorsalin sequences from the epitope insertion site (see ~~Figure 1~~ Figures 1A and 1B), were used to produce two PCR fragments, one encoding dorsalin N-terminal to the insertion site (with primer ORF-5' and the primer 5' GCGAATTCGATATCAGCTTCTGCTCTGCTCCTATGCTTCTCTTGC 3' [SEQ. ID No. 14]) and the other encoding the C-terminal region (with primer 5' CCGAATTCGATATCCGAGGAGGACCTGAACCACTGTCGGAGAACGTC 3'; SEQ --

On page 36, lines 1-3, please delete the paragraph which begins "library and to define . . ." and insert the following amended paragraph:

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--library and to define a clone containing a 3.5 kb insert with an open reading frame that encoded a protein of 427 amino acids (~~Fig. 1~~ Figures 1A and 1B).--

On page 37, lines 15-34, please delete the paragraph which begins "Medium from cells . . ." and insert the following amended paragraph:

--Medium from cells transfected with the epitope-modified *dsl-1* construct was passed over a MAb 9E10 (Evan et al., 1985) anti c-myc affinity column. Affinity purified proteins were analyzed by gel electrophoresis, revealing a major 15 kDa band and minor bands at 45,47 and -60 kDa (Fig. 3A). The bands at 45 and 47 kDa correspond in size to those predicted for the unprocessed *dsl-1* protein and the 15 kDa band to that expected for a proteolytically-cleaved product. To establish the identity of the 15 kDa band and to determine the site for proteolytic cleavage of the precursor protein, the 15 kDa band was blotted onto Immobilon membranes and subjected to sequence analysis. The NH<sub>2</sub>-terminal sequence obtained, SIGAEQKLIS (SEQ ID No. 16), corresponds to residues 319-322 of the predicted *dsl-1* sequence followed by the first 6 residues of the human c-myc epitope. This result shows that the R-S-K-R (SEQ ID No. 17) sequence at residues 315-318 is the site of proteolytic processing of the *dsl-1* precursor (arrow in ~~Fig. 1~~ Figures 1A and 1B), at least in the presence of the c-myc peptide.--

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In the Figures:

Please replace sheets 1/16 and 2/16 under the provisions of revised 37 C.F.R. §1.121 and 37 C.F.R. §1.84 with replacement sheets 1/16 and 2/16, which are attached hereto as **Exhibit A**.